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Microarticle

On the possibility of PhotoEmission Electron Microscopy for *E. coli* advanced studies

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ABSTRACT

The novel approach was proposed for detailed high-resolution studies of morphology and physico-chemical properties concomitantly at one measurement spot of *E. coli* bacterial cells culture immobilized onto silicon wafer surface in UHV conditions applying PhotoEmission Electron Microscopy under Hg lamp irradiation. For the *E. coli* characterization scanning electron microscopy (electron beam) and X-ray photoelectron spectroscopy (X-ray tube radiation) were applied prior to PhotoEmission Electron Microscopy measurements. In spite of irradiation doses collected for the cell arrays we were successful in detection of high-resolution images even of single *E. coli* bacterium by PhotoEmission Electron Microscopy technique followed by detailed high-resolution morphology studies by scanning electron microscopy. These results revealed widespread stability of the *E. coli* membranes shape after the significant number of applied characterization techniques.

The combination of a few nanometers inorganic particles with nature-like objects as a hybrid nanostructure can be a breakthrough way for the modern applications developing driven by novel low-cost and energy effective technology approaches of functional low-dimensional materials formation [1–5]. One of the convenient objects for such kind of technology development is *E. coli* bacterial cells that can be easily grown in simple and controlled laboratory conditions [6–8]. Knowledge of surface properties and composition plays an important role for such systems so the precision control is necessary to be applied. Scanning Electron Microscopy (SEM) is one of the most developed and useful tool for morphology studies of different functional materials [9–11] including biosystems such as based on *E. coli* arrays [12–15]. Nevertheless, a precise and chemically sensitive microspot surface studies on biological objects are still not well investigated field. PhotoEmission Electron Microscopy (PEEM) in coupling with laboratory or synchrotron based excitation sources can play a keyrole for such tasks [16–18]. PEEM technique provides the unique and powerful ability to perform chemically sensitive (selective) microspot spectroscopy and microscopy in one experiment run [19–21]. The crucial point for PEEM studies of bio-objects is their stability under special conditions of surface sensitive experiments.

E. coli K12 MG1665 bacteria colony were grown aerobically in LB medium at 37 °C with constant mixing up to the stationary growth phase [7] and precipitated by centrifugation (2500 rpm) then washed twice with TE-NaCl buffer solution (50 mM Tris-HCl pH 7.5; 0.1 mM EDTA; 50 mM NaCl) and twice with distilled water. The washed cells were deposited to a cleaned silicon surface (20, 40, or 60 μl), dried at 37 °C and washed three times with distilled water. For preliminary SEM studies JEOL JSM-6380LV microscope was used while for high resolution images Carl Zeiss ULTRA 55 microscope was applied. UHV X-ray photoelectron spectrometer was equipped with high resolution SPECS Phoibos 150 hemispherical electron energy analyzer with monochromatic X-ray source. Energy resolution was found in the range of 0.1 eV with the analysis depth estimation of about 3 nm. The calibration of the spectra was carried out using a signal of pure gold film and the position of Au 4f level. Omicron Focus PEEM microscope of Russian-German Lab at Helmholtz-Zentrum Berlin was used for PEEM studies.

Herein for the first time we present PEEM results for *E. coli* K12

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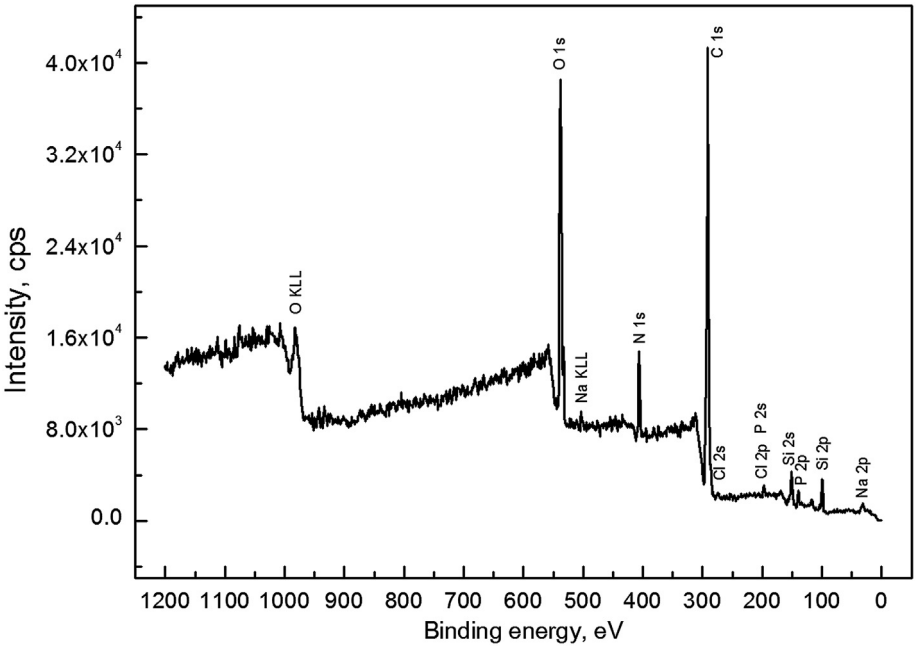

Fig. 1. XPS survey spectrum of the *E. coli* array localized on the Si wafer. Main core level lines are indicated.

MG1665 cell line in combination with X-ray photoelectron spectroscopy (XPS) control that was preceded and followed by SEM studies. SEM studies were performed to control the cells membrane morphological stability after accumulated electrons and photons radiation doses during the PEEM and XPS studies. For all consistently performed measurements the initial probes were stored for more than two months in the ambient condition being deposited on clean silicon surfaces XPS studies were performed under Al Kα radiation (1486.61 eV), SEM measurements at accelerating voltages of 2, 5 and 20 kV. Aiming at reducing the radiation damage of cells array during the PEEM measurements we used Hg lamp with excitation energy of ~5 eV.

Initial SEM images are revealed distribution of *E. coli* bacteria localized on the silicon surface with a well-known shape and other characteristics (e.g. sizes) of a single bacterium [12–14]. XPS verification spectrum (Fig. 1) confirms the absence of any noticeable surface contamination. All elements peculiar to the deposited bacterial cells arrays are observed such as carbon, nitrogen and oxygen together with buffer and working solutions residuals (Na, Cl, etc). Finally, Si core levels are detected from the substrate because of silicon substrates fragmentary coverage by *E. coli* array. All observed lines were identified at XPS spectrum given in the Fig. 1.

PEEM images (Fig. 2) demonstrate a possibility of effective bioimaging of the *E. coli* bacterial cells array using such type of visualization technique. Moreover, well distinguished observation of a single bacterium is detected by PEEM technique that correlates well with SEM results. The quality of the *E. coli* bacterial cells surface morphology features are nearly the same as compared with SEM image (Fig. 2b). SEM studies performed after XPS (X-ray irradiation) and PEEM (Hg lamp irradiation) experiments demonstrate only partial bacteria membrane destruction. Only partial destruction of the bacteria membrane confirms the surprisingly strong stability of *E. coli* MG1665 culture and its fragments. These easily observed dark parts of a single observed cells membrane can be detected in SEM and even more in PEEM images. These results suggest promising perspective of nanobiofunctional materials effective synchrotron studies [20–23] under feasibly low-dose photons excitation for microspot chemically sensitive PEEM (i.e. spectromicroscopy) surface analysis possibly performed in UHV condition that as shown in present paper can be applied up to a single *E. coli* bacterial cell bioimaging.

CRediT authorship contribution statement

S.Yu. Turishchev: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **D. Marchenko:** Methodology, Validation, Investigation, Writing - review & editing. **V. Sivakov:** Methodology, Validation, Investigation, Writing - review & editing, Funding acquisition. **E.A. Belikov:** Validation, Formal analysis, Resources. **O.A. Chuvenkova:** Methodology, Formal analysis, Investigation, Visualization. **E.V. Parinova:** Validation, Formal analysis, Investigation. **D.A. Koyuda:** Validation, Formal analysis, Investigation. **R.G. Chumakov:** Methodology, Validation, Investigation, Writing - review & editing. **A.M. Lebedev:** Methodology, Validation, Investigation, Writing - review & editing. **T.V. Kulikova:**

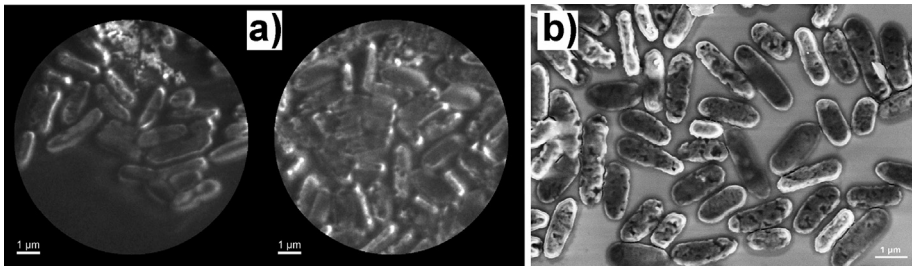

Fig. 2. High resolution PEEM images (a, two different surface areas) followed by SEM micrograph (b) of the *E. coli* array localized on the silicon substrate surface.

Methodology, Investigation. **A.A. Berezhnoy:** Methodology, Investigation, Resources. **I.V. Valiakhmedova:** Methodology, Investigation, Resources. **N.V. Praslova:** Methodology, Visualization. **E.V. Preobrazhenskaya:** Methodology, Investigation. **S.S. Antipov:** Methodology, Formal analysis, Investigation, Writing - review & editing, Supervision, Funding acquisition.

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